

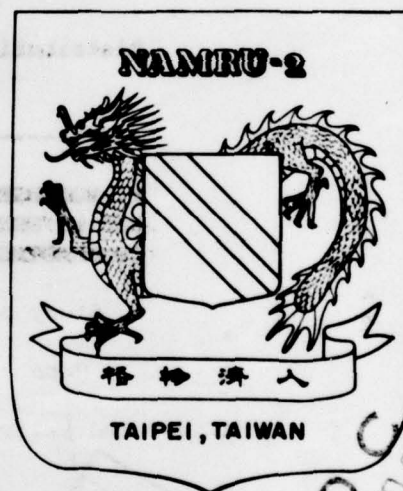
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**ARBOVIRUS ANTIBODIES IN INDONESIANS AT MALILI,  
SOUTH SULAWESI (CELEBES) AND BALIKPAPAN,  
E. KALIMANTAN (BORNEO)**

**P.F.D. VAN PEENEN, S.W. JOSEPH, J. CASALS,  
J. SULIANTI SAROSO AND R. SEE**

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**Arbovirus antibodies in Indonesians at Malili, South Sulawesi (Celebes) and Balikpapan, E. Kalimantan (Borneo)\***

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The distribution of arboviruses of medical importance in Indonesia is not widely known. We have isolated Japanese encephalitis (JE) virus from mosquitoes (Van Peenen *et al.*, 1974) and dengue types 1, 2 and 3 from patients (unpublished data) from Jakarta. In addition, we have recovered dengue types 3 and 4 from cases of haemorrhagic fever in children from Medan, North Sumatra (Van Peenen *et al.*, submitted for publication). 'Dengue haemorrhagic fever' has also been reported (without virological confirmation) from various places in Indonesia during the past three years: Padang, Central Sumatra; Bandung, West Java; Semarang and Yogyakarta, Central Java; and Surabaya, East Java (Ministry of Health, unpublished data; Rivai *et al.*, 1972; Ismangoen *et al.*, 1971). An epidemic of Chikungunya was reported from Balikpapan, East Kalimantan, in 1973 (Kanamitsu *et al.*, unpublished data).

Without proven virus isolation, knowledge of the distribution of arboviruses in Indonesia has been based mostly on the results of serological surveys. Some of them (Hotta *et al.*, 1967; Hotta, 1973) used techniques which we find difficult to interpret. More recently, Tesh *et al.* (submitted for publication) have documented the occurrence of

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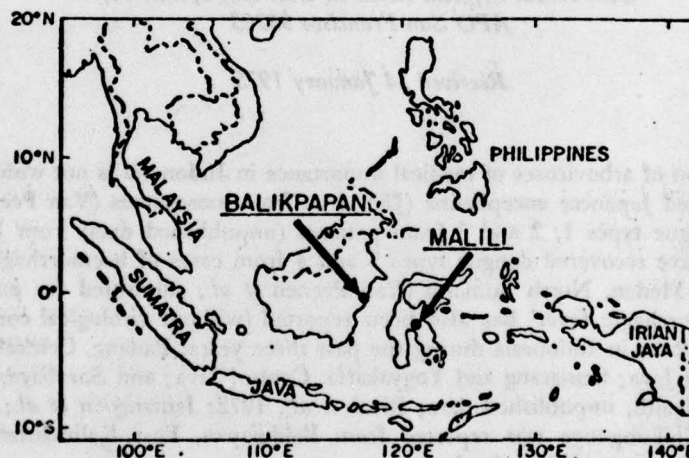


antibodies against group A arboviruses using the Plaque Reduction Neutralization Test (PRNT): antibodies were found against Chikungunya (Chik) in inhabitants of Central Sulawesi and West Kalimantan, and against Ross River virus in Indonesians from New Guinea (Irian Jaya).

This paper reports the results of analyses for several arboviruses, measured with the haemagglutination-inhibition (HI) test, on human sera obtained during surveys in South Sulawesi (Celebes) and in East Kalimantan (Borneo).

#### MATERIALS AND METHODS

Sera were collected in 1973 from the Malili area of South Sulawesi and from Balikpapan, East Kalimantan (see map). Malili is the site of a developing nickel mine, and Balikpapan is a centre of oil exploration and forestry. Malili sera came from inhabitants of local villages and from workmen in the nickel mining area; in Balikpapan, all were from workmen. We asked each individual if that person was indigenous to the area, and these and other answers were entered on computer coding forms. The sampling, however, was not random. Bleeding was done at night in different villages, and we attempted to obtain specimens from anyone available. A disproportionate number of people were probably ill. Almost 1 000 sera were obtained from the Malili area, but only 200 were selected for serological analyses. Selection was made in order to provide sera from different age groups.



Map of Indonesia showing locations sampled.

Sera were kept at  $-20^{\circ}\text{C}$  and sent by air freight to the Yale Arbovirus Research Unit (YARU) New Haven, Connecticut, U.S.A. where they were examined by HI (Theiler and Downs, 1973).

The sera were kaolin-treated and absorbed with goose erythrocytes. The antigens used were dengue types 1, 2, 3 and 4 (D1, D2, D3, D4); Japanese encephalitis (JE) virus, Nakayama strain (JE Nak), and JE Jakarta strain (JE-DJA); Yellow Fever (YF); Zika; Wesselsbron (Wess); Tembusu (Tem); Royal farm (RF); Chikungunya (Chik); Sindbis

(Sin); eastern equine encephalitis (EEE); Getah; Batai; and Ketapang (Keta). Eight units of antigen were used for most tests; occasionally 16 units were used. The 22 sera from Balikpapan were all tested at the same time and those from Malili were pooled and processed in seven separate tests. Malili sera were tested at dilutions up to 1:160 only; Balikpapan sera were tested at dilutions to 1:640 against YF, Zika, Wess, Tem and RF.

All data, including antibody titres, were entered into an IBM 1130 system available at NAMRU-2 in Taipei, and statistics done by one of us (RS).

### RESULTS

Ten villages in the Malili area were sampled. Eight were geographically adjacent and its inhabitants were of the same race. Results from these villages were grouped as 'Timampu'. Timampu (2° 39'S, 121° 25'E, alt. 295 m) is a subdistrict headquarters. A sample of 40 sera from two other villages was grouped as 'Malili'. Malili (2° 38' S, 121° 04' E, alt. 30 m) is a district headquarters. Ages and sexes for both groups are given in Table I. Fourteen persons in the Malili group, and 26 from Timampu, were not lifelong residents, but all were natives of Sulawesi. All 22 Balikpapan sera were from males: 19 aged 20-32, and one each aged 18, 19, and 54. Only one had lived all his life in Balikpapan. The others had worked there, or elsewhere in Kalimantan, for periods from one year to all their lives. Nine originally came from Java, four each from Kalimantan and Sulawesi, three from Flores Island, and in one case, the origin was not known. When first questioned, 158, (79%) of the Sulawesi subjects claimed to be sick, as did 18 of the Balikpapan group, but on further inquiry only 42 (21%) reported fever (only one in the Balikpapan group).

TABLE I

Age and sex distribution of Indonesians sampled from South Sulawesi

#### A. Timampu

Age	Sex		Total
	Male	Female	
0-9	8	3	11
10-19	18	18	36
20-29	18	16	34
30-39	20	15	35
40-49	10	8	18
50+	14	12	26
Total	88	72	160

#### B. Malili

Age	Sex		Total
	Male	Female	
0-9	1	1	2
10-19	2	4	6
20-29	7	4	11
30-39	3	4	7
40-49	2	3	5
50+	5	4	9
Total	20	20	40



*Group A, Bunyamwera, and Bakau Virus HI Antibodies*

Since HI antibodies to Batai and Keta antigens were lacking, there was little evidence of past exposure to viruses in either the Bunyamwera or Bakau groups. A 30-year-old male lifelong resident of Timampu had a titre of 1:20 against Batai, but no other antibodies.

No antibodies were detected against EEE or Getah, and only two, both from male

TABLE II  
Antibodies against group B arboviruses in sera tested

		Timampu		Malili		Balikpapan	
		No.	%*	No.	%*	No.	%*
YF	No. examined	160	(100)	40	(100)	22	(100)
	Negative	149	(93)	25	(63)	3	(14)
	1:20 - 1:40	5	(3)	14	(35)	14	(64)
	1:80 or higher	6	(4)	1	(3)	5	(23)
ZIKA	No. examined	160	(100)	40	(100)	22	(100)
	Negative	133	(83)	16	(40)	2	(9)
	1:20 - 1:40	12	(8)	9	(23)	0	(0)
	1:80 or higher	15	(9)	15	(37)	20	(91)
WESS	No. examined	160	(100)	40	(100)	22	(100)
	Negative	128	(80)	16	(40)	2	(9)
	1:20 - 1:40	16	(10)	5	(13)	1	(5)
	1:80 or higher	16	(10)	19	(47)	19	(86)
TEM	No. examined	136	(100)	35	(100)	22	(100)
	Negative	133	(98)	27	(77)	6	(27)
	1:20 - 1:40	2	(1)	7	(20)	12	(55)
	1:80 or higher	1	(1)	1	(3)	4	(18)
RF	No. examined	136	(100)	35	(100)	22	(100)
	Negative	133	(98)	31	(89)	8	(36)
	1:20 - 1:40	3	(2)	3	(9)	14	(64)
	1:80 or higher	0	(0)	1	(3)	0	(0)
JE	No. examined	160	(100)	40	(100)	22	(100)
	Negative	150	(94)	26	(65)	4	(18)
	1:20 - 1:40	6	(4)	9	(22)	13	(59)
	1:80 or higher	4	(3)	5	(13)	5	(23)
D1	No. examined	135	(100)	35	(100)	22	(100)
	Negative	118	(87)	21	(60)	2	(9)
	1:20 - 1:40	15	(11)	12	(34)	6	(27)
	1:80 or higher	2	(1)	2	(6)	14	(64)
D2	No. examined	160	(100)	40	(100)	22	(100)
	Negative	146	(91)	23	(58)	2	(9)
	1:20 - 1:40	8	(5)	14	(35)	8	(36)
	1:80 or higher	6	(4)	3	(8)	12	(55)
D3	No. examined	135	(100)	35	(100)	22	(100)
	Negative	124	(92)	21	(60)	2	(9)
	1:20 - 1:40	10	(7)	12	(34)	6	(27)
	1:80 or higher	1	(1)	2	(6)	14	(64)
D4	No. examined	135	(100)	35	(100)	22	(100)
	Negative	123	(91)	19	(54)	2	(9)
	1:20 - 1:40	10	(7)	11	(31)	3	(14)
	1:80 or higher	2	(1)	5	(14)	17	(77)

\*Percentages may not add up to 100, since all were rounded.

lifelong residents of Malili, aged 26 and 31, had low level (1:20 and 1:40, respectively) titres against Sin. Both of the latter also had antibodies against group B viruses at much higher titres. None of the Balikpapan sera had antibodies against Chik, but three of 160 (at titres of 1:20, 1:40 and 1:80) from Timampu and seven of 40 (18%) from Malili did. These seven were all from Karabbe, a village near Malili, from six males and one female, between 35 and 70 years of age. Three had titres of 1:40, three of 1:80, and one of greater than 1:160.

#### Group B

Table II summarizes the prevalences of group B arboviruses antibody titres.

Most Balikpapan sera, but relatively few of those from Sulawesi, had antibodies to dengue and/or JE. Comparison of positive results, using the two JE antigens, were usually in agreement within one tube. However, three of four Balikpapan sera, negative when tested with the Jakarta antigen, were positive when tested with the Nakayama strain antigen. Of these four, two had no antibodies against dengue, but two had titres of 1:160 or greater against all four dengue types. Only one of the Balikpapan sera had no antibody to either JE or dengue. This came from a 22-year-old Javanese who had lived in Balikpapan for more than three years.

By contrast, 150 of the 160 sera from Timampu lacked antibodies to JE, and 145 had no detectable antibodies against any of the dengue types tested. Of those positive for dengue, titres were either uniform, or sera were tested for only dengue 2, except in the case of a 32-year-old female who had lived in the area for one year only. Her serum had dengue 2 antibodies at a dilution greater than 1:160, and other dengue type antibodies at 1:40 or less.

Results from Malili sera were similar to those from Timampu. Twenty-six were negative for JE when tested with either antigen, and two were positive by only one antigen. Twenty-four had no dengue antibody titres.

Many sera had no demonstrable arbovirus antibody titres. One was in the Balikpapan group: a 22-year old male from Java who had worked in Balikpapan for more than three years. Fourteen (35%) in the Malili group had no arbovirus antibodies, and two others had only low titred (1:20) antibody levels against Wess and Zika, respectively. One hundred and twenty-seven of the Timampu sera were negative, and an additional eight had low titres against one virus only: three had titres of 1:20 and one a titre of 1:40 against dengue 1 only, one a titre of 1:20 against Wess only; one a titre of 1:20 against dengue 2 only, and one a titre of 1:20 against Batai only. Thus, 79% of Timampu sera were completely negative, and 84% were negative except against one of the viruses tested.

#### DISCUSSION

The literature on arbovirus antibodies in Indonesia is confusing, even in reports in which neutralization tests were used. Green *et al.*, (1974) reported low-titre, but positive neutralization tests, against EEE, YF, Sin and Bunyamwera in human sera collected near Jakarta in 1963. Irving *et al.* (1969), using HI, reported prevalences of JE and dengue antibodies ranging from 0 to 46% in sera from Central Java. Both Irving *et al.* (1969) and Green *et al.* (1974) reported the occurrence of Chik antibodies; the former found few by HI in Central



Java, the latter found many by neutralization tests in and near Jakarta, West Java. In fact, Green *et al.* (1974) reported that 137 of 497 Jakarta sera neutralized Chik, 55 at titres of 1:640 or higher. They also found many sera with neutralizing antibodies against EEE, YF, Sin and Bunyamwera, although highest titres in such sera were against JE. By contrast, Tesh *et al.* (submitted for publication) found no evidence of Chik neutralizing antibodies in sera from Jakarta. The presence of EEE and YF antibodies in Indonesia seems unreasonable on geographic grounds.

Hotta (1973), if we interpret his data correctly, found evidence of HI antibody titres against Chik in human sera from Lombok Island, Surabaya, East Java, Central Java (lowland), and Lampung, South Sumatra during 1964. In addition, because of differences in HI titre, he concluded that JE antibodies predominated (over dengue) in Lombok; D1 was predominant in Lampung, and D2 in Central Java.

Kanamitsu *et al.* (unpublished data), using the HI test, analysed sera from Pontianak, West Kalimantan; Balikpapan, East Kalimantan; Surabaya, East Java; Makassar, South Sulawesi; Kupang, Indonesian Timor; Ambon, Moluccas; and Jayapura, Irian Jaya. Again, if we interpret correctly, these authors found serological evidence for Chik, dengue and/or JE in all areas studied. Most serological positives for Chik were from Balikpapan; few were found in Ambon. They also found serological evidence for human Getah infection in Irian Jaya, and for Ross River infection in Pontianak, Makassar, Ambon and Irian Jaya.

By contrast, we found that the majority of indigenous Sulawesi inhabitants lacked HI antibodies to all arboviruses tested. Except for evidence of Chik infection in the Malili group, few had antibodies against Group A, Bunyamwera or Bakau groups, and none were at high titres. The 22 sera tested from Balikpapan were not from true natives and therefore cannot be considered representative of Kalimantan. Nevertheless, absence of Chik antibodies from the Balikpapan sera is surprising, since a Chik epidemic was supposed to have occurred in East Kalimantan during 1973 (Kanamitsu, unpublished data).

Chik may be present in the Malili area: 18% of the sera were positive and one had a titre of greater than 1:160. All were from seven adults aged 20-70 who were lifelong inhabitants of Karabbe. One 28-year-old male from Karabbe, and one 39-year-old male from Timampu had antibodies against Chik but none of the other arboviruses tested. The occurrence of only two sera with antibodies against Sin and one against Batai among the 222 total tested would indicate that except for Chik, other group A, Bunyamwera, and Bakau, virus groups were not present in the areas studied. These results are in agreement with those of Tesh *et al.* (submitted for publication) who found evidence of Chik neutralizing antibody in adults from Central Sulawesi. The possibility of positive Chik titres resulting from exposure to a closely related virus (as Semliki) should not, however, be ignored.

Most detectable group B HI antibodies were against JE and/or dengue, which are the only two viruses thus far isolated from Indonesia. Exceptions were in sera from two lifelong male residents of Timampu: they were 24 and 29 years of age and had titres of 1:80 and 1:40, respectively, against Zika only. An 18-year-old lifelong female resident of Malili also had a titre of 1:20 against Zika only. Antibodies against RF were found only if there were antibodies to all others in this group (Table III). Indeed, as shown in Table III, only eight of a possible 32 combinations occurred, and in all but three cases, antibodies against Wess always occurred when others were present. It therefore does not seem prudent



Table III

Group B antibody patterns exclusive of JE and dengue

Arbovirus	Number of positive sera	
	Balikpapan	Sulawesi
Negative	2 (9%)	141 (71%)
Wess only	0	8 (4%)
Zika only	0	3 (2%)
Wess + Zika	1 (5%)	20 (10%)
YF + Wess + Zika	3 (14%)	17 (9%)
Zika + Wess + Tem	0	2 (1%)
YF + Zika + Wess + Tem	2 (9%)	2 (1%)
YF + Zika + Wess + Tem + RF	14 (64%)	7 (4%)
Total	22 (100%)	200 (100%)

to speculate on the actual occurrence of these viruses in the areas studied, although from previous knowledge of virus distributions Wess and YF would not be expected.

Antibody titres against dengue did not show one type to be consistently higher than the others. Highest titres might be against any one of the four types, although titres against dengue 1 were four times greater than against other dengue types in two sera from Malili and one from Balikpapan. A four-fold higher titre against dengue 2 occurred in one each of Malili and Balikpapan sera and against dengue 4 in one Malili and three Balikpapan sera.

Royal Farm (RF) antibodies occurred in 14 of 22 Balikpapan sera, but occurred in only four of 35 sera tested from Malili and three of 136 tested from Timampu. However, other group B antibodies were always found in RF positive sera, and all RF titres were less than 1:80, except in one case from Malili with titres of 1:80 against RF but 1:160 against both Zika and Wess.

The high prevalence of HI antibodies against JE and dengue in Balikpapan sera corresponds with similarly high prevalences we have encountered in and near large cities of Java and Sumatra. Also, according to Gordon Smith *et al.* (1974), HI and neutralizing antibodies against JE and dengue were frequent in human sera from nearby Sarawak. *Aedes aegypti* and common culicine mosquitoes, including *Culex tritaeniorhynchus*, occur in Balikpapan; and pigs are raised near that city. A mosquito survey was not carried out in Malili, but pigs are present.

## SUMMARY

Two hundred human sera from South Sulawesi and 22 from East Kalimantan were analysed for arbovirus antibodies by the hemagglutination-inhibition test. Antibodies against Japanese encephalitis and dengue were present in all but one of the E. Kalimantan sera, and in many of those from S. Sulawesi. There was serological evidence of exposure to Chikungunya, and possibly Zika, virus in a few inhabitants of S. Sulawesi.

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Two hundred human sera from South Sulawesi and 22 from East Kalimantan were analysed for arbovirus antibodies by the hemagglutination-inhibition test. Antibodies against Japanese encephalitis and dengue were present in all but one of the E. Kalimantan sera, and in many of those S. Sulawesi. There was serological evidence of exposure to Chikungunya, and possibly Zika, virus in a few inhabitants of S. Sulawesi.

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